Mechanism-based inactivation of monoamine oxidases A and B by tetrahydropyridines and dihydropyridines

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1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its primary oxidation product, 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP⁺), are mechanism-based inhibitors of monoamine oxidases A and B. The pseudo-first-order rate constants for inactivation were determined for various analogues of MPTP and MPDP⁺ and the concentrations in all redox states were measured throughout the reaction. Disproportionation was observed for all the dihydropyridiniums, but non-enzymic oxidation was insignificant. The dihydropyridiniums were poor substrates for monoamine oxidase A and, consequently, inactivated the enzyme only slowly, despite partition coefficients lower than those for the tetrahydropyridines. For monoamine oxidase B, the dihydropyridiniums were more effective inactivators than the tetrahydropyridines. Substitutions in the aromatic ring had no major effect on the inactivation of monoamine oxidase B, but the 2'-ethyl- and 3'-chloro-substituted compounds were very poor mechanism-based inactivators of monoamine oxidase A. It is clear that both oxidation steps can generate the reactive species responsible for inactivation.

INTRODUCTION

In the first report showing that highly purified monoamine oxidases A and B oxidize the neurotoxic tertiary amine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), we presented evidence that MPTP is also a time-dependent irreversible inactivator of both types of monoamine oxidase [1]. Shortly thereafter we showed [2,3] (1) that not only MPTP, but also its primary oxidation product, MPDP⁺, are mechanism-based ('suicide') inhibitors of monoamine oxidases A and B, (2) that under the experimental conditions used the decay of activity on incubation of either enzyme with MPTP is exponential and is not reversed by gel exclusion or brief dialysis, but (3) that after prolonged dialysis of monoamine oxidase B previously inactivated by MPTP a part of the activity returns [3]. The mechanism-based inhibition of monoamine oxidases by MPTP and MPDP⁺ was readily distinguished from the reversible competitive inhibition of both monoamine oxidases by MPTP, MPDP⁺ and by MPP⁺, the four-electron oxidation product: the A enzyme was found to be far more sensitive to reversible inhibition by MPDP⁺ and MPP⁺ than was the B form [2,3]. The partition coefficients (the rate of product generation divided by the rate of inactivation of the enzyme) for the inactivation were large, since the rate of oxidation of MPTP by either enzyme greatly exceeded the rate of inactivation. Moreover, when [³H]MPTP was used for inactivation of the B enzyme, about 5 mol of product was incorporated/mol of protein, suggesting dissociation of the product(s) from the enzyme and spurious recombination with nucleophiles in the enzyme [3].

The time-dependent inactivation of monoamine oxidases by MPTP and MPDP⁺ has also been studied in several other laboratories. Fuller & Hemrick-Luecke [4], working with rodent brain mitochondria, independently discovered the time-depen-

dent inactivation of monoamine oxidase by MPTP, but thought that monoamine oxidase A was only reversibly inhibited by this compound. The reason why they failed to observe that the A enzyme is subject to both reversible (competitive) and irreversible (non-competitive) effects may have been due to the experimental conditions used by these workers, including the radiochemical assay based on a single time point. In contrast, Fritz *et al.* [5] and Brossi *et al.* [6], using appropriate spectrophotometric assays, confirmed the reports [1–3] that monoamine oxidase B undergoes mechanism-based inhibition by MPTP and MPDP⁺, but, curiously, claimed that MPP⁺ also produces time-dependent irreversible inactivation of monoamine oxidase B [5]. This is quite unlikely, since MPP⁺ cannot undergo further oxidation by this enzyme; moreover, the previous data contradict it [2,3]. Arai *et al.* [7,8], also reported that MPTP is a 'suicide substrate' (i.e., mechanism-based inhibitor) of monoamine oxidase B. They also observed inactivation by 4-phenyltetrahydropyridine, in conflict with the earlier report of Fritz *et al.* [5], who found no inhibition by 4-phenyltetrahydropyridine, a discrepancy probably again due to the assay procedure used by the Japanese workers [7,8], which may yield misleading kinetic data. Using appropriate initial-rate measurements, our findings were confirmed and significantly extended by Tipton *et al.* [9]. They reported a very high (17 100 ± 930) partition coefficient for the inactivation of monoamine oxidase B in rat liver by MPTP. Although this value is much higher than that documented in the present paper, it should be kept in mind that the basis of calculations differed substantially: in the work quoted [9] it was calculated from the product formed, after an extended period, when inactivation was complete; in the present study it is calculated from initial-rate measurements.

Despite the interest of many laboratories in the time-dependent inactivation by MPTP, several important questions remain

Abbreviations used: MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPDP⁺, 1-methyl-4-phenyl-2,3-dihydropyridinium species; MPP⁺, 1-methyl-4-phenylpyridinium species; 2'-Me-MPTP, 2'-Et-MPTP and 3'-Cl-MPTP, the 2'-methyl-, 2'-ethyl- and 3'-chloro-substituted analogues of MPTP respectively; 2'-Me-MPDP⁺, 2'-Et-MPDP⁺ and 3'-Cl-MPDP⁺, the 2'-methyl-, 2'-ethyl- and 3'-chloro-substituted analogues of MPDP⁺; 2'-Me-MPP⁺, 2'-Et-MPP⁺ and 3'-Cl-MPP⁺, the 2'-methyl-, 2'-ethyl- and 3'-chloro-substituted analogues of MPP⁺ respectively.

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unanswered. Thus the monophasic nature of logarithmic plots of the decrease in activity with time observed by several workers [3–5] and the similarity of the pseudo-first-order rate constants from the inactivation of monoamine oxidase B by MPTP and MPDP⁺ [2] have precluded the drawing of conclusions as to whether inactivation of the enzyme occurs during the first or the second two-electron transfer from MPTP or both. Later, the present authors found that, by varying the concentrations of MPTP or of other tetrahydropyridines and of the enzyme, biphasic kinetics become apparent. Another interesting question is the identity of the nucleophile(s) at the active site involved in covalent adduct formation, which results in the loss of activity. It has been reported [3] that proteolysis of monoamine oxidase B fully inactivated by MPTP yields a flavin peptide with a normal absorption spectrum. This seems to preclude the cysteinyl-flavin as the site of covalent attachment of an electrophilic product arising from MPTP, since adducts with the only potential nucleophilic groups on the flavin, N-5 and C-4(a), have very different absorption spectra from that of cysteinyl-FAD. Moreover, such an adduct would be expected to be stable to the treatments used in isolation of the peptide. The thiol group at the substrate site has therefore been considered a possibility [3], as in the mechanism-based inactivation by arylcyclopropylamines [10,11].

Identification of the nucleophilic group(s) responsible for the formation of the inactive adduct, in turn, required finding a tetrahydropyridine analogue with a much lower partition coefficient than the one characterizing the inactivation of monoamine oxidase B by MPTP. This would greatly increase the probability of obtaining a labelled adduct containing a nearly stoichiometric amount of inhibitor, instead of approx. 5 mol/mol, as found with MPTP [3]. Although several analogues have been tested by Gessner *et al.* [12], most failed to inactivate monoamine oxidase B; only one seemed more inhibitory than MPTP itself. The availability of a number of synthetic MPTP analogues that are good substrates of either the A or of the B enzyme or of both [13] prompted us to investigate their effectiveness as time-dependent inactivators of these enzymes.

MATERIALS AND METHODS

Monoamine oxidase A from human placental mitochondria [14] and monoamine oxidase B from ox liver mitochondria [15,16] were isolated by procedures described in the literature. Their activities were determined spectrophotometrically at 30 °C by using strict initial-rate measurements, the former with kynuramine, and the latter with benzylamine as substrate, as in previous work [3]. The active-site concentrations were based on the flavin content, which was 8 nmol/mg for monoamine oxidase A and 8.8 nmol/mg for monoamine oxidase B. The sources of

MPTP, MPDP⁺ and MPP⁺ were as in our previous studies [1,3]. All other tetrahydropyridines, dihydropyridines and pyridines used in this investigation were synthesized and their purities determined as described by Youngster *et al.* [13]. The molar absorption coefficients and the peak wavelengths of compounds used in this study are given in Table 1.

The kinetics of the inactivation were studied by incubating monoamine oxidase A or B with the tetrahydropyridine or dihydropyridium being tested at 30 °C in 0.3–1.4 ml volume. The concentrations of the enzyme and inactivator are specified in the Figure legends. Samples (5 or 10 µl, as appropriate) were removed periodically and diluted with 0.99 ml of assay mixture [3], so as to displace any reversibly bound inhibitor accumulated, and assayed immediately. Measured rates were corrected for competitive inhibition by using the Michaelis–Menten equation only in the case of monoamine oxidase A, where the diluted concentration of the inhibitor was significant compared with its K_i . Other samples (10 to 20 µl) were injected into cold 2% (w/v) HClO₄ (to give 1 ml final volume) and the concentration of each component in the sample was later determined with a Hewlett–Packard diode-array spectrophotometer and its multicomponent analysis program.

RESULTS AND DISCUSSION

Time course for the inactivation of monoamine oxidases A and B

As noted above, previous reports of the time-dependent irreversible inactivation of monoamine oxidases A and B indicated a monophasic decay of activity [3,6]. The reason for this finding was that both the concentration of the enzyme and that of the tetrahydropyridine used were high. Considering the fact that the rate of oxidation of MPTP by either form of monoamine oxidase greatly exceeds the rate of oxidation of MPDP⁺, it became apparent that the experimental conditions obscured the individual contributions of the two oxidation steps to the inactivation. Thus at high concentrations of monoamine oxidase B and moderate MPTP concentrations most of the tetrahydropyridine was oxidized before the removal of the first sample for assay: hence, during the bulk of the experiment, the inactivation was the result of MPDP⁺ oxidation. On the other hand, when the concentration of MPTP was high most of the enzyme was destroyed before the tetrahydropyridine concentration declined to a point where the contribution of MPDP⁺ oxidation to the inactivation could be detected. By using a somewhat lower concentration of enzyme and more frequent sampling, the biphasic nature of the inactivation of monoamine oxidase B by MPTP becomes apparent (Fig. 1a). Under the same conditions inactivation during the oxidation of the same concentration of MPDP⁺ is monophasic (Fig. 1b). Since the pseudo-

Table 1. Molar absorption coefficients of MPTP and of its analogues in various oxidation states

The purity of the compounds was ascertained by C, H and N analysis and n.m.r. They were dried over P₂O₅ to constant weight and dissolved in water acidified to pH 2.5 with HCl to give 20 mM concentration. Spectra were recorded with a Hewlett–Packard rapid-scan spectrophotometer. For the pyridinium compounds the blank contained equimolar KI.

Compound	Oxidation state ...		Tetrahydropyridine		Dihydropyridinium		Pyridinium	
	ϵ (mm ⁻¹ ·cm ⁻¹)	λ (nm)	ϵ (mm ⁻¹ ·cm ⁻¹)	λ (nm)	ϵ (mm ⁻¹ ·cm ⁻¹)	λ (nm)	ϵ (mm ⁻¹ ·cm ⁻¹)	λ (nm)
MPTP	13	243	17	343	19	290		
2'-Me-MPTP	25.9	192	9.84	332	8.40	288		
2'-Et-MPTP	29.1	192	9.05	326	7.88	286		
3'-Cl-MPTP	11.0	244	14.0	337	16.2	282

Fig. 1. Semi-logarithmic plot of the time-dependent inactivation of monoamine oxidase B

The enzyme, at 0.2 mg/ml protein concentration, was incubated at pH 7.2 and 30 °C with (a) 2 mM-MPTP and (b) 2 mM-MPDP⁺. Samples were removed periodically and diluted with a large excess of benzylamine to reverse any competitive inhibition by the oxidation products. The remaining activity was monitored spectrophotometrically [3].

first-order rate constant for the second phase in Fig. 1(a) (0.032 min^{-1}) is the same as the rate constant in Fig. 1(b) (0.029 min^{-1}), it seems likely that the second phase in the former experiment reflects the loss of activity occurring during the oxidation of MPDP⁺. The fact that the second phase of the inactivation initiated by MPTP is faster than the first phase (Fig. 1a), despite the lower rate of oxidation of MPDP⁺ than of MPTP by the enzyme, may be rationalized in terms of the much lower partition coefficient associated with the second than with the first phase (Table 2). As expected on theoretical grounds, the transition between the two linear phases is curved when sufficient points are sampled. The transition occurs at around 20 min after the start of the incubation, at which time (see below, Fig. 3c) MPTP and MPDP⁺ are present in approximately equal concentrations. Although the K_m values for the two compounds are similar, it was noted earlier that the K_D value for MPDP⁺ and monoamine oxidase B is at least 30-fold lower than that for MPTP [17], so that successful competition is feasible.

A biphasic time course for the mechanism-based inactivation has also been observed during the processing of various tetrahydropyridines by monoamine oxidase A. Figs. 2(a) and 2(b) are examples of this. In the case of 3'-Cl-MPTP (Fig. 2a) the rates of both phases were readily measurable and, as for MPTP, the second phase is faster. In contrast, when 2'-Me-MPTP is oxidized by monoamine oxidase A the rate of inactivation during the second phase is so low that at moderate initial substrate concentrations (1 or 2 mM) it is nearly undetectable. The higher the initial concentration of 2'-Me-MPTP added, the farther the inactivation proceeded within a limited time period (Fig. 2b). This is as expected from the relative rates of oxidation of 2'-Me-

MPTP and 2'-Me-MPDP⁺ (Table 2). The 2'-methyl analogue of MPTP is a very good substrate of monoamine oxidase A but has a very high partition coefficient, so that if added at low concentration most of it is oxidized before extensive inactivation can occur, whereas 2'-Me-MPDP⁺ is a very poor substrate so that it cannot cause significant inactivation within the time frame of the experiment, despite its lower partition coefficient (Table 2). It may be noted in this connection that 2'-Me-MPDP⁺ was assayed at a much higher enzyme concentration (3.9 mg/ml) than 2'-Me-MPTP (0.26 mg/ml). These conditions were selected because of constraints imposed by the properties of the system. 2'-Me-MPTP is oxidized so fast that a low protein concentration had to be used to detect any inactivation before the substrate was fully oxidized, but the slow oxidation of 2'-Me-MPDP⁺ by the enzyme necessitated the use of a much higher enzyme concentration.

It is clear from these experiments that the use of tetrahydropyridines as mechanism-based inactivators of monoamine oxidases A and B offers a unique opportunity to extend our understanding of this important class of enzyme inhibitors, because of the two consecutive oxidase steps involved, each of which may result in the formation of an irreversible adduct with the enzyme.

The system, however, presents difficulties in the measurement of the kinetics and the partition coefficients of the individual events. First, inactivation during the oxidation of the tetrahydropyridine and of the dihydropyridinium often overlap during a substantial part of the experiment. Secondly, both the dihydropyridinium and pyridinium products are good competitive inhibitors, particularly of monoamine oxidase A, so that conditions

Table 2. Mechanism-based inactivation of monoamine oxidases A and B by tetrahydropyridines and dihydropyridines

Experiments were carried out at pH 7.2 at 30 °C with 2 mM substrate, except with 2'-Et-MPTP and monoamine oxidase A, where the substrate concentration was 1 mM. Protein concentration was 0.2 mg/ml for monoamine oxidase B but was varied from 0.2 to 3 mg/ml for monoamine oxidase A. Values in parentheses are uncertain because of extensive disproportionation during the experiment. Note that $v_{app.}$ is not a true turnover number, because the enzyme concentration was high, which results in lower observed activity for this enzyme.

Inhibitor	Concn. of protein (mg/ml)	$v_{app.}$ (μ mol/min per μ mol of enzyme)	$k_{inact.}$ (min^{-1})	Partition coefficient ($v_{app.}/k_{inact.}$)
Monoamine oxidase A				
MPTP	0.2	20	0.0103	1950
MPDP ⁺	1.0	(0.74)	(0.0043)	(172)
2'-Me-MPTP	0.26	88	0.0477	1834
2'-Me-MPDP ⁺	3.9	0.7	0.0057	120
2'-Et-MPTP	0.2	32	0.006	5400
2'-Et-MPDP ⁺	1.26	1.57	0.007	229
3'-Cl-MPTP	3.0	20	0.0077	2590
Monoamine oxidase B				
MPTP	0.2	24	0.0187	2406
MPDP ⁺	0.2	(3.0)	0.027	(111)
2'-Me-MPTP	0.2	84	0.026	3220
2'-Me-MPDP ⁺	0.2	8.7	0.0178	486
2'-Et-MPTP	0.2	71	0.036	1980
2'-Et-MPDP ⁺	0.2	(7.1)	0.0182	(390)
3'-Cl-MPTP	0.2	64	0.0231	2790
3'-Cl-MPDP ⁺	1.0	2.0	0.0309	65

Fig. 2. Semi-logarithmic plot of the time-dependent inactivation of monoamine oxidase A

Experimental conditions were as in Fig. 1, except that a high concentration of monoamine oxidase A was used during incubation [3 mg/ml in the experiment of (a) and 8 mg/ml in that of (b)] and samples were taken for assay with kynuramine under the standard assay conditions. (a) Inactivation by 2 mM 3'-Cl-MPTP; (b) inactivation by 2'-Me-MPTP. The concentrations were: $\square$, 1 mM; $\bullet$, 2 mM; $\circ$, 3 mM. $\blacksquare$ symbols are the control incubated without tetrahydropyridine.

must be selected to assure that the product carried over in the samples taken for assay is not present at such a high concentration that the substrate used in the assay cannot displace it; otherwise the remaining activity will not be fully expressed. Thirdly, the dihydropyridinium, besides undergoing both enzymic and non-enzymic oxidation [3], also disproportionates, yielding more of the tetrahydropyridine [18]. For all of these reasons it is necessary to know the concentration of the pyridine compound in each of the three oxidation states throughout the course of the inactivation. This may be ascertained by the use of a diode-array spectrophotometer and an appropriate multi-component analysis program, as illustrated in Fig. 3.

Fig. 3(a) shows the absorption spectra of MPTP, MPDP⁺ and MPP⁺; the last is corrected for the contribution of I⁻ added with

the MPP⁺. Fig. 3(b) is the experimentally observed spectrum during the inactivation of monoamine oxidase B by MPTP in the experiment of Fig. 1(a) at 17 min. Fig. 3(c) shows the concentration of the individual components during the course of the experiment of Fig. 1(a), calculated by the multi-component analysis program, based on the three spectra in Fig. 3(a). Comparing Figs. 1(a) and 3(c), it is seen that the loss of activity, except during the first few minutes, is due to both MPTP and MPDP⁺ oxidation and that during the latter part of the experiment most of the inactivation is due to the processing of MPDP⁺.

This technique does not resolve another problem in the determination of the partition coefficient with some MPDP⁺ analogues. With compounds that are only very slowly oxidized,

Fig. 3. Multi-component analysis by rapid-scan spectrophotometry of the products formed during inactivation of monoamine oxidase B

(a) Absorption spectra of MPTP (—), MPDP⁺ (.....), and MPP⁺ (-----). (b) Observed spectrum during the inactivation of monoamine oxidase by 2 mM-MPTP. The spectrum was taken after 17 min incubation in the experiment of Fig. 1(a). (c) Concentration of the individual components during the inactivation in the experiment of Fig. 1(a) derived by multi-component analysis. ▲, MPTP; ■, MPDP⁺; ●, MPP⁺.

the substrate concentration may have to be raised to such high levels that chemical disproportionation is excessive and comparable with the enzyme-catalysed rate. Disproportionation is suppressed at pH values below 7, but in this range the enzyme may be unstable. Monoamine oxidase A is particularly susceptible to both of these sources of error, especially with the very slowly oxidized dihydropyridinium compounds (Table 2).

Disproportionation and non-enzymic oxidation

Evidence has been presented [3] that at low concentrations of MPDP⁺ (approx. 50 μ M) and at pH 7.2, conditions under which the disproportionation of MPDP⁺ is negligible, non-enzymic oxidation of MPDP⁺ to MPP⁺ occurs at measurable rates. The question arose whether such autooxidation of MPDP⁺ contributes significantly to the formation of MPP⁺ at the much higher MPDP⁺ concentrations used in this study (2 mM), particularly with dihydropyridinium analogues that disproportionate faster than MPDP⁺ itself, such as 3'-Cl-MPDP⁺. Fig. 4 shows that,

Fig. 4. Disproportionation of 2 mM-3'-Cl-MPDP⁺ at pH 7.2

Conditions: 50 mM-phosphate buffer, pH 7.2 at 30 °C. ○, 3'-Cl-MPDP⁺; △, 3'-Cl-MPTP; □, 3'-Cl-MPP⁺.

since at no time does the formation of 3'-Cl-MPP⁺ exceed that of 3'-Cl-MPTP, autooxidation cannot be detected in these conditions. Further, the sum of the two accounts for the decline in 3'-Cl-MPDP⁺ concentration within experimental error.

Fig. 4 also serves to illustrate two additional observations. First, at least with this analogue, which disproportionates more rapidly than the other analogues tested, the process can be quite extensive, leading to the disappearance of half of the 2 mM-dihydropyridinium within 30 min. This mandates that experiments on the mechanism-based inactivation of monoamine oxidases by MPDP⁺ and its analogues be of short duration, so as to minimize the build-up of the tetrahydropyridine, which would then compete for processing by the enzyme. The second observation is that at pH 7.2 (but not at and below pH 6.5) disproportionation seems to follow pseudo-first-order kinetics, though over a brief initial time interval the rate of appearance of the pyridinium approximates linearity (Fig. 4). At pH 6.0 the disproportionation was second-order, as expected. For the sake of simplicity, values for the rate of disproportionation were calculated from measurements of the appearance of the pyridinium during the interval when no more than 5–10% of the initial dihydropyridinium concentration has disappeared. With the use of this approximation of the rate of disproportionation, the appearance of MPP⁺ from MPDP⁺ at 30 °C (at the concentration given in parenthesis) was as follows: at pH 6.0, (0.4 mM) 0.024, (0.8 mM) 0.080 and (2.0 mM) 0.564 nmol/ml per min; at pH 6.5, (0.4 mM) 0.117, (0.8 mM) 0.363 and (2.0 mM) 1.43 nmol/ml per min; at pH 7.0, (0.8 mM) 1.67 and (2.0 mM) 3.48; at pH 7.2, (2.0 mM) 4.54 nmol/min per ml. It is clear that both the pH and the concentration of the dihydropyridinium have major effects on the apparent rate of disproportionation.

Substitution in the aromatic ring also has a major effect on the disproportionation of MPDP⁺ analogue (Fig. 5). For the compounds used in this study at a concentration of 2 mM the relative rates in nmol/ml per min were: MPDP⁺, 4.54 < 2'-Me-MPDP⁺, 8.35 < 2'-Et-MPDP⁺, 15.6 < 3'-Cl-MPDP⁺, 21.0.

Fig. 5. Comparison of the rates of disproportionation of MPDP⁺ analogues

Experimental conditions were as in Fig. 4 and all analogues were present initially at 2 mM concentration. Disproportionation is shown in terms of rates of formation of the corresponding pyridinium compounds, although each sample was analysed for all three components. ○, 3'-Cl-MPP⁺; △, 2'-Et-MPP⁺; □, 2'-Me-MPP⁺; ×, MPP⁺. The broken lines are linear extrapolations of the initial rates.

Mechanism-based inactivation of monoamine oxidases A and B by tetrahydropyridines and dihydropyridines

Table 2 summarizes the characteristics of the mechanism-based inactivation of monoamine oxidases by the tetrahydropyridines and dihydropyridinium compounds used in this study. In evaluating the data, it is important to keep in mind that the rates given here (v_{app}) are not catalytic-centre activities, in part because considerably higher protein concentration had to be used than in the determination of turnover numbers, in part because the rates are not V_{max} values with respect to the substrate. This is reflected in the ratio of the rates of oxidation of MPTP by monoamine oxidase B to that by monoamine oxidase A, shown as 2.3 in Table 2, whereas the true ratio of catalytic centre activities is about 10 [13]. (An earlier paper [3] reported a ratio of 15, but this was expressed per mg of protein, rather than molarity of the enzyme, and the preparation of monoamine oxidase A used in that study was only about 70–75% pure.) To a lesser extent the values reported in Table 2 for the rate of inactivation and the partition coefficient may vary somewhat depending on the concentration of enzyme and substrate used, so that these values should be regarded as pertaining to the prevailing experimental conditions. The conclusions drawn below are nevertheless considered to be valid under a variety of experimental conditions.

First, dihydropyridiniums seem to be very poor substrates of monoamine oxidase A and, consequently, inactivate the enzyme only very slowly. In contrast, although considerably more slowly oxidized also by monoamine oxidase B than the corresponding tetrahydropyridines, they are relatively effective inactivators. In some cases, e.g. MPDP⁺ and 3'-Cl-MPDP⁺, the observed rates of

inactivation were faster and the partition coefficients lower than for the parent tetrahydropyridines. Secondly, the nature of the substituent in the aromatic ring does not seem to have a major effect on the observed rate of inactivation of monoamine oxidase B by MPTP analogues, although all three analogues tested gave a significantly higher rate of inactivation than MPTP itself. In contrast, the observed rate of inactivation of monoamine oxidase A by 2'-Me-MPTP was over 4 times faster than that by MPTP itself, but 2'-Et-MPTP and 3'-Cl-MPTP proved to be poor mechanism-based inactivators. Thirdly, although the partition coefficients for the inactivation of either type of monoamine oxidase by some dihydropyridiniums were much lower than we [3] or others [9] previously observed with MPTP itself [3,9], none of the compounds yielded a value approaching unity, which would be required for chemical characterization of an adduct formed with the inactivated enzyme.

In the final analysis, the mechanism-based inactivation of monoamine oxidases by MPTP and its analogues has brought to light some interesting aspects of the phenomenon for cases where the enzyme undergoing inactivation catalyses sequential reactions, each of which yields an electrophilic product and, hence, a potential inactivator. This type of 'suicide substrate' poses inherent limitations to further explorations of the detailed events, in part because the products of both oxidation steps are potent competitive inhibitors especially of monoamine oxidase A, in part because the dihydropyridinium formed in the first step is subject to disproportionation, thus complicating kinetic analysis.

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